WO 2017/044672 discloses a cellular platform for the screening of functional T cell receptors. This platform is based on a murine thymoma-derived cell line that is TCR negative and CD8 positive. To facilitate screening of human TCRs, the cell line is optionally equipped with a chimeric CD8 variant that is composed of the mouse CD8 transmembrane and intracellular regions and the human CD8 extracellular region. In addition, the cell line can further comprise a reporter system that provides an indication of TCR activity. To screen for functional TCRs, the cells are transduced with retroviral vectors comprising genes encoding chimeric TCRs that are composed of a human variable region fused to a murine non-variable region. The main disadvantage of this platform is that the genes encoding the TCRs randomly integrate into the genome of the cell line, including possible multiple TCR integrations per cell. This has the consequence that expression of the TCR by the cell line can significantly vary and thereby bias the screening results.

Accordingly, there is a need for a cellular platform that is not affected by T cell activation or exhaustion status and allows facile and high throughput functional screening of TCRs, wherein, preferably, the TCRs are homogenously expressed from a single locus.

SUMMARY

In one aspect, the present disclosure refers to an isolated cell derived from a T cell, the cell comprising one or more reporter systems, wherein the endogenous T cell receptor (TCR) alpha gene locus and/or beta gene locus of the cell is modified such that surface expression of a TCR-CD3 complex is disrupted.

In another aspect, the present disclosure refers to a method for producing a cell expressing a TCR-CD3 complex on the cell surface, the method comprising the steps of: a) providing a cell wherein the endogenous T cell receptor (TCR) alpha gene locus and/or beta gene locus of the cell is modified such that surface expression of a TCR-CD3 complex is disrupted; b) introducing a DNA fragment comprising coding sequences encoding a TCR alpha variable domain, a TCR beta variable domain and at least one of a TCR alpha constant domain and/or a TCR beta constant domain into the genome of the cell of step (a); c) expressing the coding sequences encoded on the DNA fragment introduced in step (b); and d) obtaining a cell expressing a TCR-CD3 complex on the cell surface.

In another aspect, the present disclosure refers to a library of DNA fragments, wherein the library comprises DNA fragments comprising a coding sequence encoding a TCR alpha variable domain, a coding sequence encoding a TCR beta variable domain and at least one of a coding sequence encoding a TCR alpha constant domain and/or a coding sequence encoding a TCR beta constant domain; in particular a) wherein the coding sequences encoding the TCR alpha variable

domain, the TCR beta variable domain and at least one of the TCR alpha constant domain and/or the TCR beta constant domain have been obtained by sequencing TCR genes in a population of T cells, in particular, wherein the population of T cells comprises tumor-infiltrating lymphocytes, in particular wherein the coding sequences encoding the TCR alpha and beta variable domains comprised in at least one DNA fragment of the library have been obtained from the same T cell; or b) wherein at least one DNA fragment in the library comprises one or more mutations in at least one coding sequence encoding a TCR alpha variable domain, a TCR beta variable domain and at least one of a TCR alpha constant domain and/or a TCR beta constant domain compared to a DNA fragment with a known sequence; in particular wherein the one or more mutations have been introduced into the coding sequences encoding the complementarity determining regions (CDRs) of the variable domains of the T cell receptor, in particular wherein the one or more mutations have been introduced into the coding sequences encoding the CDR3 of the variable alpha and/or variable beta domains of the T cell receptor; and /or wherein the one or more mutations have been introduced into the DNA fragments by deep mutational scanning.

In another aspect, the present disclosure refers to a method for determining the potential of a cell expressing a T cell receptor to bind to and/or to be activated by an antigen, the method comprising the steps of: a) producing a cell expressing a T cell receptor using the method as disclosed herein; b) contacting the cell of step (a) with an antigen, in particular wherein the antigen is part of an antigen library; and c) determining the potential of the cell to bind to the antigen and/or to be activated by the antigen; in particular wherein the cell is determined to bind to the antigen if the binding between the cell and the antigen is stronger compared to the binding between the cell and an irrelevant antigen, or, if the binding between the cell and the antigen is stronger compared to the binding between the antigen and a cell that expresses an irrelevant or no T cell receptor; and/or wherein the cell is determined to be activated by the antigen, if the activation of the cell by the antigen is higher compared to the activation of a cell that has been contacted with an irrelevant or no antigen, or, if the activation of the cell by the antigen is higher compared to the activation of a cell that expresses an irrelevant or no T cell receptor and has been contacted with the antigen.

In another aspect, the present disclosure refers to a method for identifying at least one T cell receptor with optimized antigen-binding or antigen-induced signaling properties, the method comprising the steps of: a) producing a plurality of cells using the method as disclosed herein, wherein the plurality of cells is produced by introducing the library of DNA fragments as disclosed herein into said cells; b) contacting the plurality of cells of step (a) with a target antigen or with one or more non-target antigens; c) isolating at least one cell from the plurality of cells, in which the binding between the cell and the target antigen and/or in which the activation of the cell by the target antigen is increased compared to a cell that expresses a T cell receptor that has been used as starting point for the optimized T cell receptor; and/or, in which the binding between the cell and the non-target antigen and/or in which the activation of the cell by the non-target antigen is decreased compared to a cell that expresses a T cell receptor that has been used as a starting point for the optimized T cell receptor; and d) identifying at least one T cell receptor with optimized antigen-binding or antigen-induced signaling properties by sequencing the DNA fragment comprised in the at least one cell isolated in step (c); in particular

wherein the non-target antigen is a peptide that is presented by an APC or by an MHC multimer, in particular wherein the peptide is pulsed onto the APC or wherein the peptide is genetically encoded by the APC, or wherein the non-target antigen is a cell that does not present the target antigen on its cell surface.

In yet another aspect, the present disclosure refers to a method for identifying at least one antigen-specific T cell receptor, the method comprising the steps of: a) producing a plurality of cells using the method as disclosed herein, wherein the plurality of cells is produced by introducing the library of DNA fragments as disclosed herein into said cells; b) contacting the plurality of cells of step (a) with an antigen; c) isolating at least one cell from the plurality of cells, in which the binding between the cell and the antigen of step (b) is stronger compared to the binding between the cell and an irrelevant antigen, or, in which the binding between the cell and the antigen is stronger compared to the binding between the antigen and a cell that expresses an irrelevant or no T cell receptor; and/or in which the activation of the cell by the antigen of step (b) is higher compared to the activation of a cell that has been contacted with an irrelevant or no antigen, or, in which the activation of the cell by the antigen is higher compared to the activation of a cell that expresses an irrelevant or no T cell receptor and has been contacted with the antigen; and d) identifying at least one antigen-specific T cell receptor by sequencing the DNA fragment comprised in the at least one cell isolated in step (c).

DETAILED DESCRIPTION

The technical problem is solved by the embodiments provided in the claims. That is, the present invention relates to the following items:

1. An isolated cell derived from a T cell, the cell comprising one or more reporter systems, wherein the endogenous T cell receptor (TCR) alpha gene locus and/or beta gene locus of the cell is modified such that surface expression of a TCR-CD3 complex is disrupted.

2. The cell according to item 1, wherein the isolated cell is derived from a human or murine T cell.

3. The cell according to any one of items 1 or 2, wherein the isolated cell is derived from a Jurkat T cell.

4. The cell according to any one of items 1 to 3, wherein the cell further comprises a co-receptor.

5. The cell according to item 4, wherein the co-receptor is encoded by genes that are operably linked to their corresponding promoter or to a recombinant promoter.

CLAIMS

1. An isolated cell derived from a T cell, the cell comprising one or more reporter systems, wherein the endogenous T cell receptor (TCR) alpha gene locus and/or beta gene locus of the cell is modified such that surface expression of a TCR-CD3 complex is disrupted.

2. The cell according to claim 1, wherein the isolated cell is derived from a human or murine T cell, in particular wherein the isolated cell is derived from a Jurkat T cell.

3. The cell according to claim 1 or 2, wherein the cell further comprises a co-receptor in particular wherein the co-receptor is CD4 or CD8.

4. The cell according to claim 3, wherein the co-receptor is encoded by genes that are operably linked to their corresponding promoter or to a recombinant promoter, in particular wherein the recombinant promoter is a constitutive promoter.

5. The cell according to any one of claims 1 to 4, wherein the one or more reporter system comprises a polynucleotide comprising a promoter region operably linked to a polynucleotide sequence encoding a detectable marker.

6. The cell according to claim 5, wherein the promoter region comprises one or more transcriptional response elements, in particular wherein at least one of the one or more transcriptional response elements is the nuclear factor of activated T cell (NFAT) response element.

7. The cell according to claim 5 or 6, wherein the promoter region comprises 3 NFAT response elements.

8. The cell according to any one of claims 1 to 7, wherein the detectable marker is a fluorescent protein, in particular wherein the fluorescent protein is a green fluorescent protein (GFP).

9. The cell according to any one of claims 1 to 8, wherein expression and/or activity of

a) at least one receptor involved in programmed cell death or T cell exhaustion is reduced

or disrupted; in particular

wherein the receptor involved in programmed cell death or T cell exhaustion is a Fas receptor, TNFR2 and/or PD-1, in particular, wherein the receptor is a Fas receptor; and.or

b) at least one protein involved in antigen presentation is reduced or disrupted, in particular wherein the protein is beta-2 microglobulin; and/or

c) at least one protein promoting non-homologous end joining is reduced or disrupted, in particular wherein the protein is p53 or p53-binding protein 1; and/or

d) at least one protein promoting homology directed repair is increased, in particular, wherein the protein is BRCA1.

10. The cell according to any one of claims 1 to 9, wherein the cell comprises a gene encoding a recombinant endonuclease, in particular wherein the recombinant endonuclease is *Streptococcus pyogenes* Cas9.

11. A method for producing a cell expressing a TCR-CD3 complex on the cell surface, the method comprising the steps of:

a) providing a cell wherein the endogenous T cell receptor (TCR) alpha gene locus and/or beta gene locus of the cell is modified such that surface expression of a TCR-CD3 complex is disrupted;

b) introducing a DNA fragment comprising coding sequences encoding a TCR alpha variable domain, a TCR beta variable domain and at least one of a TCR alpha constant domain and/or a TCR beta constant domain into the genome of the cell of step (a);

c) expressing the coding sequences encoded on the DNA fragment introduced in step (b); and

d) obtaining a cell expressing a TCR-CD3 complex on the cell surface.

12. The method according to claim 11, wherein the DNA fragment of step (b) further comprises homology arms at its 5’ and 3’ ends that are homologous to the endogenous TCR alpha locus or the endogenous TCR beta gene locus of the cell, in particular wherein the DNA fragment further comprises a splice donor site that is located downstream of the most 3’ coding sequence of the DNA fragment and upstream of the 3’ homology arm.

13. The method according to claim 11 or 12, wherein the DNA fragment is introduced into the genome of the cell such that the coding sequences located on the DNA fragment replace the

endogenous TCR alpha VJ exon or the TCR beta VDJ exon and are transcriptionally linked to the endogenous gene encoding the TCR alpha constant domain or the TCR beta constant domain, respectively; and/or

wherein the DNA fragment is introduced into the genome of the cell through homologous recombination, homology-directed repair, homology-independent targeted insertion or microhomology-mediated end joining, in particular wherein the DNA fragment is introduced into the genome of the cell through CRISPR-Cas9-mediated homology-directed repair.

14. A library of DNA fragments, wherein the library comprises DNA fragments comprising a coding sequence encoding a TCR alpha variable domain, a coding sequence encoding a TCR beta variable domain and at least one of a coding sequence encoding a TCR alpha constant domain and/or a coding sequence encoding a TCR beta constant domain; in particular

a) wherein the coding sequences encoding the TCR alpha variable domain, the TCR beta variable domain and at least one of the TCR alpha constant domain and/or the TCR beta constant domain have been obtained by sequencing TCR genes in a population of T cells, in particular, wherein the population of T cells comprises tumor-infiltrating lymphocytes, in particular wherein the coding sequences encoding the TCR alpha and beta variable domains comprised in at least one DNA fragment of the library have been obtained from the same T cell; or

b) wherein at least one DNA fragment in the library comprises one or more mutations in at least one coding sequence encoding a TCR alpha variable domain, a TCR beta variable domain and at least one of a TCR alpha constant domain and/or a TCR beta constant domain compared to a DNA fragment with a known sequence; in particular wherein the one or more mutations have been introduced into the coding sequences encoding the complementarity determining regions (CDRs) of the variable domains of the T cell receptor, in particular wherein the one or more mutations have been introduced into the coding sequences encoding the CDR3 of the variable alpha and/or variable beta domains of the T cell receptor; and /or wherein the one or more mutations have been introduced into the DNA fragments by deep mutational scanning.

15. A method for determining the potential of a cell expressing a T cell receptor to bind to and/or to be activated by an antigen, the method comprising the steps of:

a) producing a cell expressing a T cell receptor using the method according to any one of claims 11 to 13;

b) contacting the cell of step (a) with an antigen, in particular wherein the antigen is part of an antigen library; and

c) determining the potential of the cell to bind to the antigen and/or to be activated by the antigen;

in particular wherein the cell is determined to bind to the antigen if the binding between the cell and the antigen is stronger compared to the binding between the cell and an irrelevant antigen, or, if the binding between the cell and the antigen is stronger compared to the binding between the antigen and a cell that expresses an irrelevant or no T cell receptor;

and/or

wherein the cell is determined to be activated by the antigen, if the activation of the cell by the antigen is higher compared to the activation of a cell that has been contacted with an irrelevant or no antigen, or, if the activation of the cell by the antigen is higher compared to the activation of a cell that expresses an irrelevant or no T cell receptor and has been contacted with the antigen.

16. A method for identifying at least one T cell receptor with optimized antigen-binding or antigen-induced signaling properties, the method comprising the steps of:

a) producing a plurality of cells using the method according to any one of claims 11 to 13, wherein the plurality of cells is produced by introducing the library of DNA fragments according to claim 14 into said cells;

b) contacting the plurality of cells of step (a) with a target antigen or with one or more non-target antigens;

c) isolating at least one cell from the plurality of cells, in which the binding between the cell and the target antigen and/or in which the activation of the cell by the target antigen is increased compared to a cell that expresses a T cell receptor that has been used as starting point for the optimized T cell receptor;

and/or,

in which the binding between the cell and the non-target antigen and/or in which the activation of the cell by the non-target antigen is decreased compared to a cell that expresses a T cell receptor that has been used as a starting point for the optimized T cell receptor; and

d) identifying at least one T cell receptor with optimized antigen-binding or antigen-induced signaling properties by sequencing the DNA fragment comprised in the at least one cell isolated in step (c);

in particular wherein the non-target antigen is a peptide that is presented by an APC or by an MHC multimer, in particular wherein the peptide is pulsed onto the APC or wherein the peptide is genetically encoded by the APC, or wherein the non-target antigen is a cell that does not present the target antigen on its cell surface.

17. The method according to claim 16, wherein the at least one identified T cell receptor is included in a training set for a machine learning algorithm to predict T cell receptors with improved antigen-binding or antigen-induced signaling properties.

18. A method for identifying at least one antigen-specific T cell receptor, the method comprising the steps of:

a) producing a plurality of cells using the method according to any one of claims 11 to 13, wherein the plurality of cells is produced by introducing the library of DNA fragments according to claim 14 into said cells;

b) contacting the plurality of cells of step (a) with an antigen;

c) isolating at least one cell from the plurality of cells, in which the binding between the cell and the antigen of step (b) is stronger compared to the binding between the cell and an irrelevant antigen, or, in which the binding between the cell and the antigen is stronger compared to the binding between the antigen and a cell that expresses an irrelevant or no T cell receptor;

and/or

in which the activation of the cell by the antigen of step (b) is higher compared to the activation of a cell that has been contacted with an irrelevant or no antigen, or, in which the activation of the cell by the antigen is higher compared to the activation of a cell that expresses an irrelevant or no T cell receptor and has been contacted with the antigen; and

d) identifying at least one antigen-specific T cell receptor by sequencing the DNA fragment comprised in the at least one cell isolated in step (c).

19. The method according to claim 18, wherein the antigen is presented on the surface of a tumor cell, in particular wherein the tumor cell and the polynucleotide sequences of the TCR coding sequences in the library of DNA fragments have been obtained from the same subject.

20. The method according to any one of claims 15 to 18, wherein the antigen or the target antigen is a peptide bound to a major histocompatibility complex (MHC), in particular wherein the peptide is presented by an MHC on the surface of an antigen-presenting cell (APC) or by an MHC multimer, in particular wherein the peptide is pulsed onto the APC or wherein the peptide is genetically encoded by the APC; and/or

wherein the binding between an antigen and a cell expressing a T cell receptor is determined by measuring the avidity of the T cell receptor for the antigen, in particular wherein the antigen is a peptide bound to a fluorescently-labeled MHC multimer; in particular wherein the avidity is measured by flow cytometry; and/or

wherein the activation of a cell expressing a T cell receptor is determined by measuring the expression of the reporter system or by measuring the expression of an activation marker after contact with an antigen, in particular wherein the antigen is a peptide presented on the surface of an APC; in particular wherein the activation marker is CD69.